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Supplemental information

Molecular mechanism of interactions between ACAD9 and binding partners in mitochondrial respiratory complex I assembly

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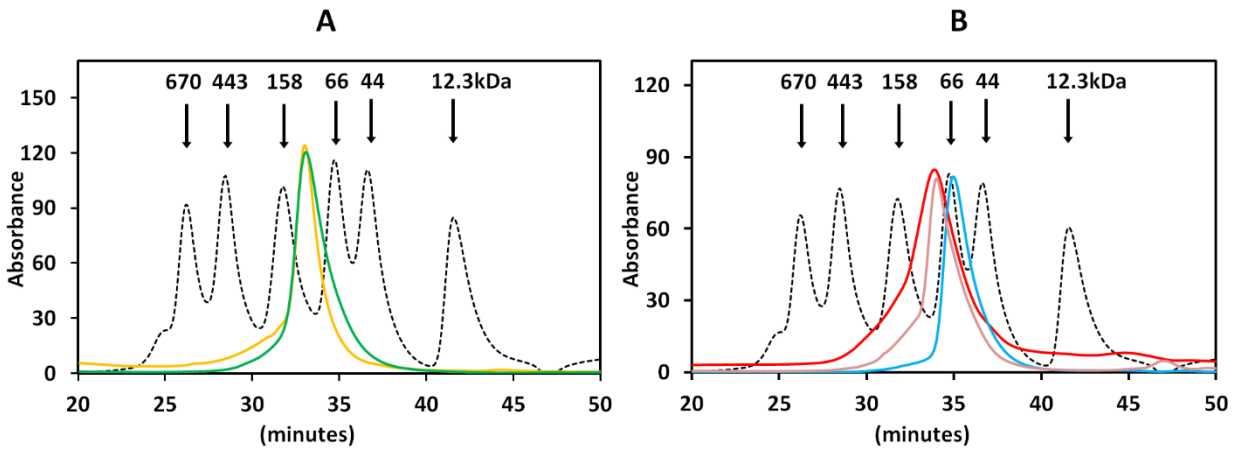

Figure S1: Purification of ACAD9, VLCAD, ECSIT, C-ECSIT, and NDUFAF1 by size exclusion chromatography (SEC). Related to STAR Methods.Elution profiles from a SEC column (BioRad Enrich SEC 650) are shown. The standard MW protein peaks (grey dashed curves) from left to right include thyroglobulin (670kDa), apoferritin (443kDa), IgG (158kDa), bovine serum albumin (67kDa), ovalbumin (43kDa), and cytochrome *c* (12.3kDa). A. Overlay of ACAD9 (green) and VLCAD (gold). B. Overlay of ECSIT (red), C-ECSIT (pink) and NDUFAF1 (cyan).

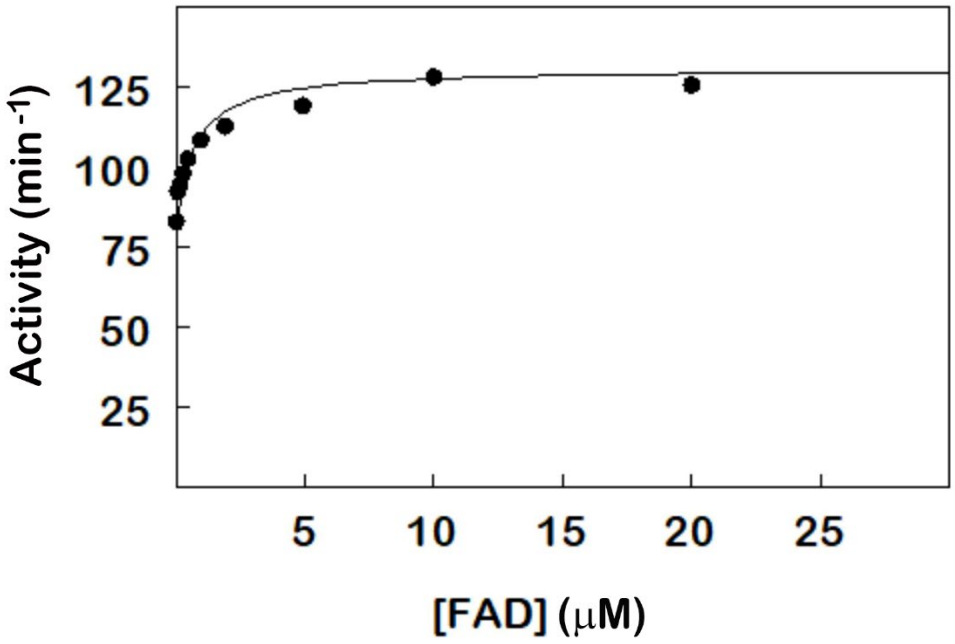

Figure S2: Stimulation of dehydrogenation activity of ACAD9 by exogenous FAD. Related to Table 1. Purified ACAD9 was incubated with the indicated FAD concentrations prior to assay as described in Methods. [FAD] is the final concentration in the assay. The solid line is a fit of the data to the equation (y=(a0*x)/(a1 + x)) + a2, where a0 is the maximum stimulated activity (48 units), a1 is the concentration of FAD at which half-maximal stimulation of activity was observed (0.75 μM), and a2 is the activity observed in the absence of exogenous FAD (83 units, corresponding to ~63% holoenzyme).

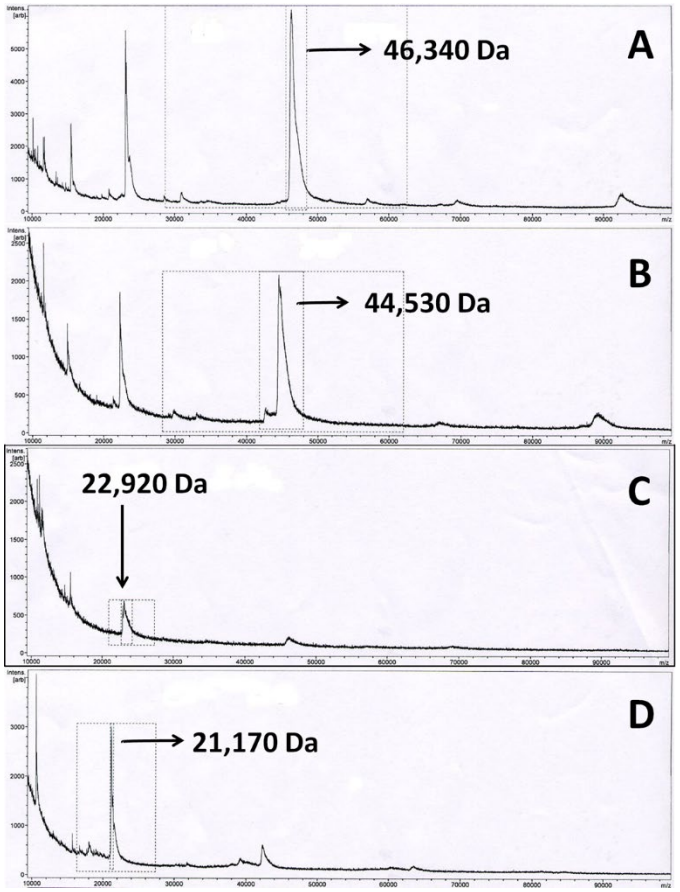

Figure S3: ECSIT MALDI-TOF mass spectra. Related to STAR Methods. The calculated molecular weights of His_6-ECSIT and His_6-C-ECSIT before and after thrombin cleavage are 46,353, 44,570, 22,960 and 21,177 Da, respectively. **A.** His_6-ECSIT spectrum before thrombin cleavage of His-tag. B. His_6-ECSIT spectrum after thrombin cleavage of His-tag. C. His_6-C-ECSIT before thrombin cleavage of the His-tag. D. His_6-C-ECSIT after thrombin cleavage of the His-tag.

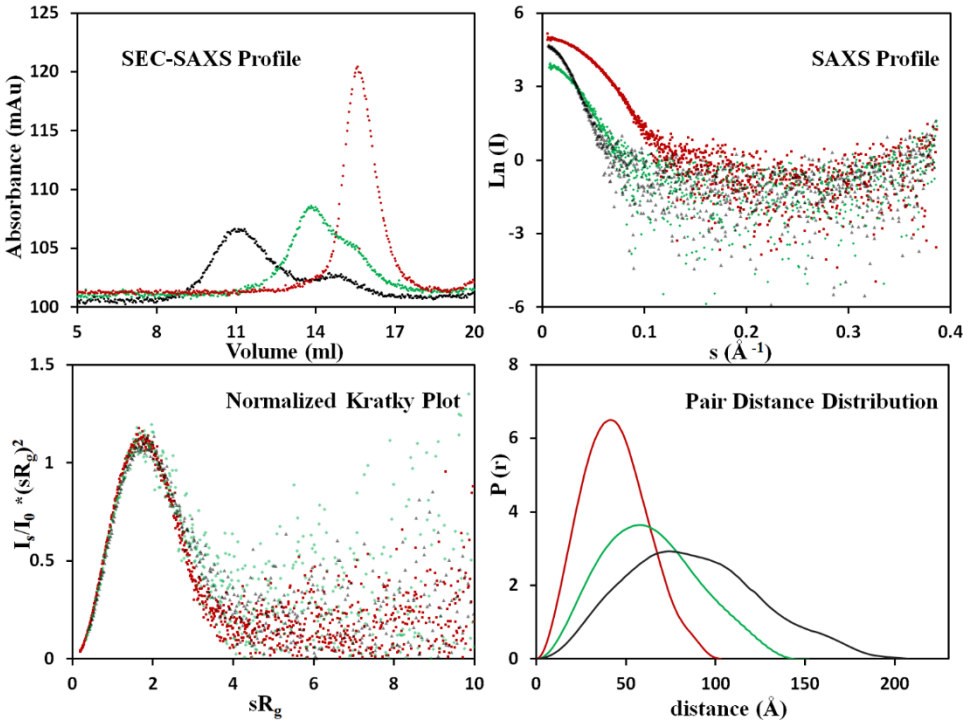

Figure S4: SEC-SAXS characterization of ACAD9, ACAD9/ECSIT binary complex, and ACAD9/ECSIT/NDUFAF1 ternary complex. Related to Table 2. ACAD9 is shown in red, the ACAD9/ECSIT binary complex in green, and the ACAD9/ECSIT/NDUFAF1 ternary complex in gray. Kratky plots indicate all three molecules are globular. The Pair distance distribution plots show the Dmax values of 100 Å, 140 Å, and 200 Å for ACAD9, the binary complex, and ternary complex, respectively.

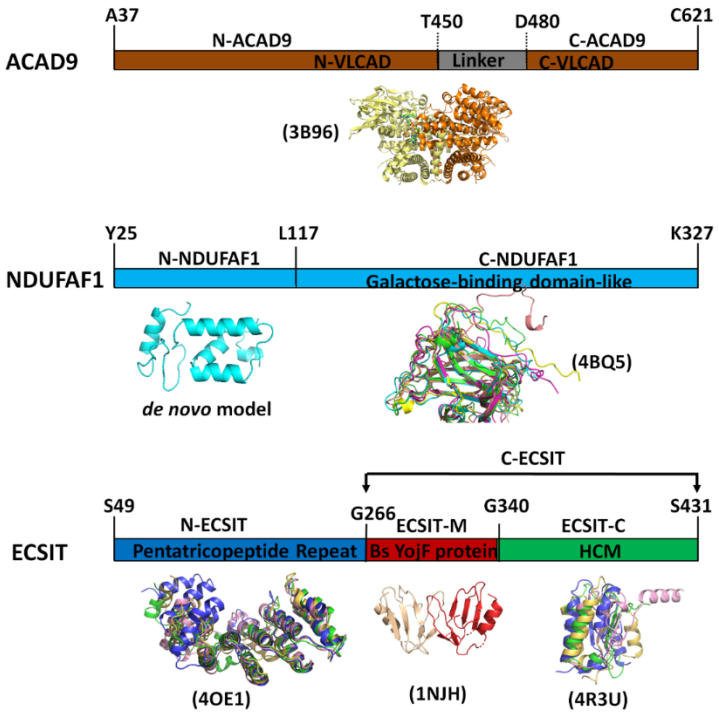

Figure S5: ACAD9, NDUFAF1, and ECSIT Domain Organization and modeled structures derived from Robetta. Related to Figure 4. C-ECSIT is now subdivided into ECSIT-M and ECSIT-C.

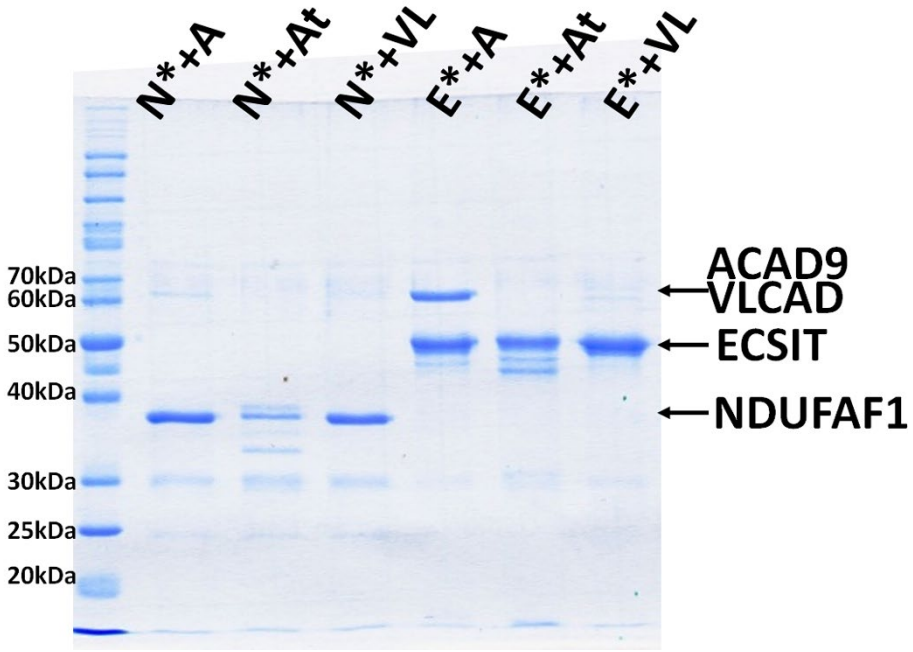

Figure S6: Ni-NTA Pull-Down Assays with His-tagged NDUFAF1 or ECSIT. Related to Figure 1. N*,His_6-NDUFAF1; A, ACAD9; At, ACAD9 subjected to limited trypsin digestion; VL, VLCAD;E*, His_6-ECSIT. Positions of full-length ACAD9, VLCAD, His_6-ECSIT, and His_6-NDUFAF1 are indicated by arrows. Molecular weights are indicated on the left. Pulldown assays were carried out as described in Methods. Limited trypsin digestion was performed as described previously [Schiff, M., et al. (2015). Hum Mol Genet *24*, 3238-3247], yielding ~45, ~48, and ~17 kDa peptides.